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10/617,060	07/10/2003	Peter H. Kay	47675-52	2645

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EXAMINER

GOLDBERG, JEANINE ANNE

ART UNIT	PAPER NUMBER
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1634

DATE MAILED: 09/13/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/617,060

Applicant(s)

KAY, PETER H.

Examiner

Jeanine A. Goldberg

Art Unit

1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 05 July 2006.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-17 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-17 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

1. This action is in response to the papers filed July 5, 2006. Currently, claims 1-17 are pending.
2. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow. This action is made FINAL.
3. Any objections and rejections not reiterated below are hereby withdrawn.

Maintained Rejections

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

4. Claims 1-6, 14-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Elsas, II et al. (US Pat. 6,207,387, March 27, 2001) in view of either Ehrlich et al. (Biochimica et Biophysica Acta, Vol. 395, pages 109-119, 1975) or Hua et al. (Gov. Rep. Announce. Index US, Vol. 88, No. 18, Abstract No. 847,050 1988) and in further view of either Tyagi et al (US Pat. 6,150,097, November 2000) or Coull et al (US Pat. 6,355,421, March 2002).

Elsas, II et al. (US Pat. 6,207,387, March 27, 2001) teaches detecting mutations in genes by determining the melting temperature of the hybrid of the amplified DNA and the specific oligonucleotide (col. 10, lines 20-25). Elsas teaches that "under identical conditions, two strands that are not exactly complementary, differing by even one nucleotide, will be less stable and will dissociate at a temperature which exactly complementary hybrids remain paired (col. 8, lines 50-57). Elsas teaches that the melting temperature between a mismatched hybrid will denature at a lower temperature than a exact matched hybrid (col. 10, lines 37-40). Elsas also teaches that fluorescence energy transfer is a specific application of this approach (col. 10, lines 49-50). Elsas teaches the different melting temperatures for the allele specific probe and detection of fluorescence (col. 11).

While Elsas teaches detecting different nucleic acids based upon melting temperature, Elsas does not specifically teach the structure of fluorescence energy transfer and does not teach using the fluorescence energy transfer for detecting methylation.

Erlach teaches Xanthomonas phage XP-12 DNA containing 5-methylcytosine completely replacing cytosine, has the highest reported melting temperature for any naturally occurring DNA (abstract). The melting temperature is 6.1 degrees Celsius higher than normal DNA containing the same percentage of adenine plus thymine (abstract), page 114). As seen in Figure2, the XP-12 DNA has a higher melting temperature (page 114).

Similarly, Hua teaches that the melting temperature of methylated Z-DNA is 387K which is 7K higher than a similar calculation for unmethylated B-DNA which is in agreement with observation (abstract).

Moreover, Tyagi et al. (herein referred to as Tyagi) teaches using nucleic acid hybridization probes having a first conformation when not interacting with a target and a second conformation when interacting with a target and having the ability to bring a label pair into touching contact in one formation but not the other (abstract). Tyagi teaches using quenching molecules and other fluorophores as efficient quenching moieties for fluorophores when attached to nucleic acid hybridization probes (col 3, lines 40-43)(limitations of Claim 2). The probes of Tyagi contain a hairpin structure which comprise single stranded loop of the hairpin and two arm sequences which form a double stranded stem hybrid (col. 5, lines 10-15). Tyagi teaches that the molecular beacon probes may have target recognition sequences 7-140 nucleotides in length (col 5, lines 24-25)(limitations of Claims 3-6). Additionally Tyagi teaches the arms that form a stem hybrid or stem duplex are 3-25 nucleotides in length (col. 5, lines 26-27)(limitations of Claim 16). Tyagi teaches a kit which contains a hairpin probe with labels (col 19, lines 66-67, Claim 12)(limitations of Claim 18).

Coull et al. (herein referred to as Coull) teaches methods of detecting target sequences using a probe which has a measurable change in one property of at least one donor or acceptor moiety of the probe which can be used to detect, identify or quantitated the target sequence in a sample. As seen in Figure 11, configuration III, a probing segment is flanked on either side by a arm segment and either a quencher and

fluorophore. The hairpin loop and stem structure allows energy transfer between donor and acceptor moieties linked at opposite ends of the nucleic acid polymer (col. 7, lines 30-37). The probing segments is designed to hybridized to at least a portion of a target sequence (col. 8, lines 35-36). In the method of Coull, a sample is contacted with the molecular beacon and a change in detectable signal associated with at least one donor or acceptor moiety of the probe is detected, identified or quantitated. Coull teaches that the assay may be used to detect a target sequence which is specific for a genetically based disease including cancer. Coull teaches that the probing sequence hybridizes to the entire target sequence (col. 16, lines 20-25). The probing sequence will generally have a length between 5-30 units in length (col. 16, lines 32-34)(limitations of Claims 3-6). The arm segments are 2-6 units in length (limitations of Claim 16). Coull teaches that shorter probes are less costly to synthesize, are generally easier to purify and should exhibit few non-specific interactions since they will comprise less nucleobase sequence diversity (col. 20, lines 29-32)(limitations of Claim 15). Coull teaches kits which comprise one or more PNA Molecular Beacons (col. 24, lines 51-67)(limitations of Claim 18). Coull teaches considerable analysis of the T_m melting temperature for the stem-loop hairpin probes (col. 20, 37-44). Coull teaches that the probes exhibit a low inherent noise (background) and an increase in detectable signal upon binding of the probe to a target sequence (col. 7, lines 40-42).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have modified the method of Elsas for detecting different nucleic acids based upon different hybridization properties including melting temperatures with the teachings

of either Tyagi or Coull which discuss and describe stem-loop and fluorescence energy transfer with the teachings of either Ehrlich or Hua teaching the properties of methylated DNA. Detecting nucleic acids based upon different melting temperatures and dissociation properties was used to identify mismatches in nucleic acids. Both Ehrlich and Hua teach that methylated DNA and unmethylated DNA have different melting temperatures. The ordinary artisan would have recognized based upon the teachings that in the art, namely Ehrlich and Hua, that in addition to mismatched DNA, methylation could also be detected based upon different melting temperature and dissociation rates. Combining the teachings of Elsas and either Ehrlich or Hua would yield an assay which would detect methylated nucleic acids as compared to unmethylated nucleic acids. Detection of methylated nucleic acids as compared to unmethylated nucleic acids is of interest to the clinical diagnostics because numerous genes are methylated in cancer as compared to unmethylated in normal tissue. Therefore, combining the teachings of Elsas and Ehrlich or Hua, a hybridization assay for differentiation methylated DNA from unmethylated DNA does not require the use of enzymes, solid supports would facilitate the detection of methylation in genes and may be used as an indicator for cancer. Moreover, the use of hairpin stem-loop probes with fluorophores and quenchers for detecting target nucleic acids in samples is taught by both Tyagi and Coull would have provided a fluorescent detection assay which is easily detectable in a single tube which does not require subjection to a gel or solid support. The use of FRET allows for the direct detection of nucleic acid target sequences without the requirement that labeled nucleic acid hybridization probes or primers be separated from the hybridization

complex prior to detection (Coull et al. col. 1, lines 45-50). Therefore, using the specific teachings about fluorescence energy transfer techniques, as described in Coull and Tyagi, would facilitate the fluorescence energy transfer method for detecting different nucleic acids as taught by Elsas. Therefore, given all of the teachings well known in the art, at the time the invention was made, a FRET-like method for detection of different nucleic acids based upon the known property that methylated and unmethylated DNA molecules have different hybridization properties would have been obvious to the ordinary artisan.

Response to Arguments

The response traverses the rejection. The response asserts that the examiner has failed to make out a prima facie case of obviousness. The response asserts that the mere observation by Ehrlich and Hua that melting temperatures of methylated DNA sequences can be higher than melting temperatures of non-methylated DNA sequences is not a teaching or suggestion to modify the method of Elsas from detecting nucleic acid substitutions in a sample to detecting methylated nucleic acids in a sample. This argument has been reviewed but is not convincing because the art at the time recognized that differences in nucleic acids may be characterized by different melting temperatures as exemplified by Elsas. Thus, the ordinary artisan would have been motivated to have detected additional differences in nucleic acids using the same technique of melting temperature properties, such as differences in methylated and nonmethylated nucleic acids.

The response asserts that the skilled artisan would not have any reasonable expectation that the two different types of mutations would have the same melting behavior. This argument has been thoroughly reviewed, but is not found persuasive because both Erlich and Hua teach the differences in melting temperature of methylated and unmethylated DNA. Thus, the ordinary artisan would have had a reasonable expectation of success to use the different melting behavior of methylated and unmethylated DNA to distinguish between the nucleic acids.

The response filed July 2006 indicates that the cited references teach completely methylated sequences on both strands and comparisons between DNAs having different structures. This argument has been reviewed but is not persuasive because the claims are not limited to non-completely methylated sequences with different structures moreover. The response further asserts that Elsas teaches at least one mismatch is associated with a significant effect on T_m . However the methylated nucleic acids would not have T_m differentials sufficient to enable effective detection. This argument has been reviewed but is not deemed persuasive because Erlich teaches a difference in T_m of 6.1 C and Hua teaches a difference of 7K . The attorney arguments indicating that the differential effect would be too small is not persuasive. MPEP 716.01(c) makes clear that "The arguments of counsel cannot take the place of evidence in the record. In re Schulze , 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965). Examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration include statements regarding unexpected results, commercial success, solution of a long - felt need, inoperability of

the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant." Here, the statements regarding the inoperability of the prior art must be supported by evidence, not argument.

The response asserts that none of the references teach the element of detecting methylated nucleic acids or the element of an oligonucleotide sequence containing a region that is susceptible to methylation. This argument has been thoroughly reviewed, but is not found persuasive because the combination of the references which demonstrate detection of nucleic acids based upon different melting temperatures, the observation that methylated and unmethylated nucleic acids have different temperatures suggests that methylated nucleic acids may be detected based upon different melting temperatures.

Thus for the reasons above and those already of record, the rejection is maintained.

5. Claims 7, 10, 12, 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Elsas, II et al. (US Pat. 6,207,387, March 27, 2001) in view of either Ehrlich et al. (Biochimica et Biophysica Acta, Vol. 395, pages 109-119, 1975) or Hua et al. (Gov. Rep. Announce. Index US, Vol. 88, No. 18, Abstract No. 847,050 1988) and in further view of either Tyagi et al (US Pat. 6,150,097, November 2000) or Coull et al (US Pat. 6,355,421, March 2002) as applied to Claims 1-6, 14-17 above, and further in view of Herman et al. (US Pat. 6,265,171, July 2001).

The combination of Elsas, Ehrlich or Hua and Tyagi or Coull does not specifically teach detecting methylation in GSTpi or calcitonin which is differentially expressed in cancer versus a normal state.

However, Herman et al. (herein referred to as Herman) teaches numerous genes which are differentially methylated at CpG islands in neoplastic versus normal tissue (limitations of Claim 7). These genes include GSTpi and calcitonin (limitations of Claims 10, 12). Herman also teaches that CpG island differential methylation may be detected in prostate cancer (col. 112, Claim 12)(limitations of Claim 12). Aberrant methylation in the 5' promoter of E-cadherin is prostate, breast and many other carcinomas (col. 27, lines 5-10).

Therefore, using the method of Elsas, Ehrlich or Hua and Tyagi or Coull in view of the teachings of differential methylation in glutathione-S-transferase-II(pi) and calcitonin. The ordinary artisan would have been motivated to have detected methylation in these two specific genes because Herman teaches that they contain methylated CpG neoplastic versus normal tissue.

Response to Arguments

The response traverses the rejection. The response asserts that since the combination of the references fails to render independent claim 1 obvious the combination does not render the claims obvious. The response asserts that Herman only teaches "the desire to detect methylation of CpG because the presence of methylated CpG in a 5' regulatory region can be indicative of a cell proliferative disorder." This argument has been reviewed but is not convincing because the Herman

has only been relied upon to the extent that the response agrees with the examiner. As provided above, the rejection of Elsas, Ehrlich or Hua and Tyagi or Coull has been maintained, therefore, the ordinary artisan would have been motivated to have used the method to have detected differential cancer methylation as taught by Herman. Thus for the reasons above and those already of record, the rejection is maintained.

6. Claims 7, 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Elsas, II et al. (US Pat. 6,207,387, March 27, 2001) in view of either Ehrlich et al. (Biochimica et Biophysica Acta, Vol. 395, pages 109-119, 1975) or Hua et al. (Gov. Rep. Announce. Index US, Vol. 88, No. 18, Abstract No. 847,050 1988) and in further view of either Tyagi et al (US Pat. 6,150,097, November 2000) or Coull et al (US Pat. 6,355,421, March 2002) as applied to Claims 1-6, 14-17 above, and further in view of Kay et al (Leukemia and Lymphoma, Vol. 24, pages 211-220, 1997).

The combination of Elsas, Ehrlich or Hua and Tyagi or Coull does not specifically teach detecting methylation in Myf-3 which is differentially expressed in cancer versus a normal state.

However, Kay et al. (herein referred to as Kay) teaches the Myf-3 gene is abnormally hypermethylated in non-Hodgkins lymphoma (abstract).

Therefore, using the method of Elsas, Ehrlich or Hua and Tyagi or Coull in view of the teachings of differential methylation in glutathione-S-transferase-II(pi) and calcitonin. The ordinary artisan would have been motivated to have detected

methylation in these two specific genes because Herman teaches that they contain methylated CpG neoplastic versus normal tissue.

Response to Arguments

The response traverses the rejection. The response asserts that since the combination of the references fails to render independent claim 1 obvious the combination does not render the claims obvious. The response asserts that Kay only teaches "that the Myf-3 gene can be hypermethylated and that the hypermethylated status of the Myf-3 gene may provide new diagnostic indicators of malignancy." This argument has been reviewed but is not convincing because the Herman has only been relied upon to the extent that the response agrees with the examiner. As provided above, the rejection of Elsas, Ehrlich or Hua and Tyagi or Coull has been maintained, therefore, the ordinary artisan would have been motivated to have used the method to have detected differential cancer methylation as taught by Herman. Thus for the reasons above and those already of record, the rejection is maintained.

New Grounds of Rejection Necessitated by Amendment

New Matter

7. Claims 1-17 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

In the amended claims, namely Claim 1, reference to "sufficient spatial proximity" is included. The amendment does not provide any identification of support in the specification for the newly added limitations. However, the specification does not describe or discuss the stem-loop arrangement requiring any "sufficient" spatial proximity. Instead the specification teaches that the stem-loop and quenching of the fluorophore moiety when in spatial proximity. This description does not support "sufficient spatial proximity". The concept of "sufficient spatial proximity" does not appear to be part of the originally filed invention. Therefore, "sufficient spatial proximity" constitutes new matter.

In the amended claims, namely Claims 9, 11, reference to "methylated CpG-containing variants" of SEQ ID NO: 1-5 are included. The amendment does not provide any identification of support in the specification for the newly added limitations. However, the specification does not describe or discuss "methylated CpG-containing variants" of SEQ ID NO: 1-5. Instead the specification describes SEQ ID NO: 1-5. This description does not support "methylated CpG-containing variants" of SEQ ID NO: 1-5. The description of SEQ ID NO: 1-5 does not provide support for variants of these sequences. The concept of "methylated CpG-containing variants" of SEQ ID NO: 1-5 does not appear to be part of the originally filed invention. Therefore, "methylated CpG-containing variants" of SEQ ID NO: 1-5 constitutes new matter.

Applicant is required to cancel the new matter in the reply to this Office Action.

Conclusion

8. No claims allowable.

9. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

A) Collins (J. Mol. Biol. Vol. 198, pages 737-744, 1987) teaches that base modifications such as methylation are known to affect the melting temperature of DNA and may be evaluated using electrophoresis.

B) Leng et al. (Biochimica et Biophysica Acta Vol. 174, pages 574-585, 1969) teaches in comparison to native DNA, the melting temperature of methylated DNA is decreased.

C) Yamasaki et al. (Proc. Japan Acad. Vol. 74, Ser. B, pages 210, 1998) teaches methylation of four adenine bases in a decamer DNA duplex decreased the melting temperatures by 9.4 degrees.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 7:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached on (571) 272-0735.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

The Central Fax Number for official correspondence is (571) 273-8300.

Art Unit: 1634

A handwritten signature in black ink, reading "J. Goldberg". The signature is fluid and cursive, with the first letter "J" being large and stylized.

Jeanine Goldberg

Primary Examiner

September 6, 2006